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Serological and Genomic Characterization of Human Rotaviruses Detected in China

Huixia Wu, Koki Taniguchi,* Tomoko Urasawa, and Shozo Urasawa
Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan

A total of 1,385 stool specimens were collected from children with diarrhea at two hospitals in Wuhan, Hubei Province, China, in 1994 and 1995, and screened for rotavirus by polyacrylamide gel electrophoresis of viral RNA. Group A rotavirus was detected with high frequency; 56.5% (87/154) and 40.8% (502/1,231) of the specimens collected in 1994 and 1995, respectively, were positive for rotavirus. Assignment of G serotype and P type (VP4 genotype) of group A rotavirus by ELISA with monoclonal antibodies and/or PCR, respectively, showed that strains of G2-P[4] and G1-P[8] specificity were predominant in 1994 and in 1995, respectively. In contrast, a single strain was found to have a P[9] type specificity, and no G4 strain was detected. Unusual combinations of RNA pattern-subgroup-G serotype-P type, such as long pattern-subgroup I-G1-P[8], short pattern-subgroup II-G3-P[4] and short pattern-subgroup I-G1-P[4], were detected in four specimens. Nucleotide sequences of the VP8* and/or NSP5 genes from two Chinese P[8] strains 470 and 582 and one Chinese P[9] strain 512 as well as five Japanese P[9] strains (K8, AU1, M318, O264, and O265) were determined and compared with the published sequences of the corresponding gene. In the phylogenetic tree of VP8* sequences of P[9] strains, which formed two clusters each having strain K8 or AU-1 as the representative strain, the Chinese P[9] strain was found in the cluster represented by AU-1, although it was most distantly related to other strains. While NSP5 sequences of human strains with P[9] specificity were related to simian and bovine strains, that of Chinese P[8] strains was most closely related to those of porcine strains. A single group C rotavirus (No. 208) was detected. Nucleotide sequences of its VP4, VP6, VP7, and NSP4 genes were very similar to those of group C human rotaviruses detected worldwide. ***J. Med. Virol. 55: 168–176, 1998.***

KEY WORDS: group A human rotavirus; group C human rotavirus; sequence; NSP5 gene; serotype; phylogeny

INTRODUCTION

Rotavirus is a major cause of acute non-bacterial gastroenteritis in infants and young children and in the young of most avian and mammalian species worldwide [Kapikian and Chanock, 1996]. On the basis of their antigenic properties and RNA profiles in polyacrylamide gel electrophoresis (PAGE), rotaviruses are classified into groups A to F [Estes, 1996]. Group A rotavirus is known to have the highest prevalence and pathogenicity.

Rotaviruses have 11 segments of double-stranded RNA (dsRNA) as a genome which is enclosed in a trilayered protein capsid. The outer capsid consists of two independent neutralization antigens VP4 and VP7 associated with P serotype and G serotype specificity, respectively. While at least 14 different G serotypes (G1 to G14) have been established among human and animal rotaviruses, G1 to G4 are the major G serotypes in humans. In contrast, typing of gene 4 alleles (VP4 genotyping) has been used as a proxy method for P serotyping, since any satisfactory serological reagent for P serotyping is not available and since the VP4 genotype determined by comparative amino acid sequence analysis is well correlated with the P serotype determined by serological methods. At least 20 VP4 genotypes (P types) have been reported [Estes, 1996]. It is recommended that when both the P serotype and genotype are shown, P genotype number included in a square bracket is affixed after the P serotype. In general, P1A[8], P1B[4], P2[6] and P3[9] predominate in human rotavirus. Surveys of G serotype and P type distribution of rotavirus at a global level have presented data for evaluating vaccine field trials, and provided clues for exploring the ecology and evolution of rotavirus. Indeed, previous studies on serotype distribution of rotavirus and on overall relatedness of viral genome by RNA-RNA hybridization of strains strongly suggested the cross-species transmission between hu-

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*Correspondence to: Koki Taniguchi, Department of Hygiene, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo 060, Japan.

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TABLE I. Detection of Rotaviruses and Their RNA Pattern in the Fecal Specimens Collected in Wuhan, China

Year of collection	No. of specimens	No. of specimens positive for rotavirus (%)	No. of specimen with long RNA pattern (%)	No. of specimens with short RNA pattern (%)	No. of specimens with long and short RNA patterns
1994	154	87 (56.5%)	23 (26.4%)	59 (67.8%)	5 (5.7%)
1995	1231	502 (40.8%)	498 (99.2%)	4 (0.8%)	0 (0.0%)

mans and animals. However, only a small number of specimens positive for rotavirus have been characterized to date in China. In this study, a number of rotavirus strains detected in China were thoroughly characterized by PAGE, enzyme-liked immunoassay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and nucleotide sequencing.

MATERIALS AND METHODS

Collection of Stool Specimens

Stool specimens were collected from children (1 month to 8 years of age) with acute gastroenteritis who were admitted to two hospitals (Wuhan Children Hospital and Tongji Medical University Hospital) in Wuhan, China. One hundred fifty-four and 1,231 specimens were obtained in 1994 and 1995, respectively. They were stored at –20°C until use.

Viruses and Cells

Approximately 10% stool suspension was made in phosphate-buffered saline, clarified by low-speed centrifugation (2,000*g* for 20 min), and kept at –20°C until use. For isolation of human rotaviruses in cell culture and propagation of cultivatable human and animal rotavirus strains, MA-104 cell were employed [Urasawa et al., 1981]. Although primary monkey kidney cells have been described to support more efficient rotavirus isolation directly from fecal specimens [Ward et al., 1984], we could not employ these due to lack of availability. For plaque isolation, CV-1 cells were used, since much clearer plaques were produced in these cells [Taniguchi et al., 1994].

PAGE

Viral RNA was extracted from a stool suspension with a disruption solution containing sodium dodecyl sulfate (SDS), 2-mercaptoethanol and EDTA, and then with phenol-chloroform. Extracted RNA was precipitated with ethanol. The RNA was electrophoresed in 10% acrylamide gels (2-mm thick) for 16 hr at 20 mA at room temperature. RNA segments were visualized by silver staining.

ELISA for Subgrouping and G Serotyping

ELISA with subgroup-specific and G serotype-specific monoclonal antibodies (MAbs) was carried out as described previously [Taniguchi et al., 1987; Urasawa et al., 1989]. The following MAbs were used: group A-common YO-156 (directed to VP6), subgroup I-specific S2-37 (VP6), subgroup II-specific YO-5 (VP6), G1-specific KU-4 (VP7), G2-specific S2-2G10 (VP7), G3-specific YO-1E2 (VP7), G4-specific ST-2G7 (VP7), and group A-common YO-2C2 (VP4).

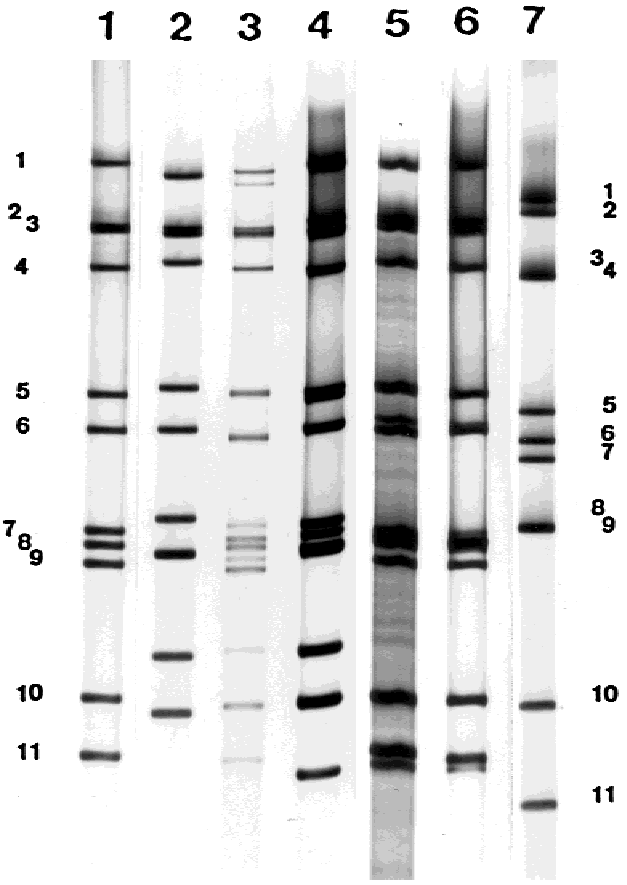

Fig. 1. RNA profiles of group A human rotaviruses showing mixed infection and/or RNA rearrangement and a group C human rotavirus. Lane 1, strain KU; lane 2, strain DS-1; lane 3, specimen No. 090; lane 4, specimen No. 114; lane 5, specimen No. A407; lane 6, specimen No. 490; lane 7, specimen No. 208.

RT-PCR for G Serotyping and P Typing

RT-PCR was undertaken in two steps (first and second amplifications) as described previously [Taniguchi et al., 1992; Wu et al., 1994]. In the first amplification for VP7-PCR, complementary DNA corresponding to the full-length VP7 gene was amplified with a pair of primers corresponding to the common 3′ and 5′ ends of the VP7 gene. The second amplification was carried out with a mixture of primers specific to each of the variable regions of VP7 genes of G1, G2, G3, G4, G8, and G9 which were paired with a primer to the common 3′ end of the VP7 gene. For VP4-PCR, a pair of primers

TABLE II. Subgroup and G Serotype Distribution of Human Rotaviruses in Wuhan, China, in 1994 and 1995 as Determined by ELISA and PCR

Year	Subgroup	No. of specimens	G serotype					
			G1	G2	G3	G4	ND	NT
1994	I	58	3 (1)	50 (12)	0	0	5 (5)	0
	II	7	2	0	4	0	1 (1)	0
	I + II	2	0	0	0	0	1 (1)	1 (1)
	ND	8	2	0	6 (4)	0	0	0
	NT	11	11 (11)	0	0	0	0	0
	Total	86	18 (12)	50 (12)	10 (4)	0	7 (7)	1 (1)
1995	I	2	0	2	0	0	0	0
	II	136	118 (9)	0	11	0	7 (7)	0
	I + II	5	2 (2)	0	1 (1)	0	2 (2)	0
	ND	60	50 (15)	0	7 (3)	0	3 (3)	0
	NT	0	0	0	0	0	0	0
	Total	203	170 (26)	2	19 (4)	0	12 (12)	0

The numbers in the parentheses indicate the number of specimens whose G serotype was determined by PCR.
PCR was applied to 67 specimens (25 in 1994 and 42 in 1995) whose G serotype could not be determined by ELISA and to 11 specimens for which ELISA could not be carried out due to the shortage of the specimen.
ND, could not be determined by ELISA for subgroup, and could not be determined by either ELISA or PCR for G serotype.
NT, not tested.

corresponding to the common sequence of nucleotide nos. 11 to 32 and nos. 1,072 to 1,094 were used for the first amplification, and a mixture of primers specific to each of the variable regions of P1A[8], P1B[4], P2[6], and P3[9] which were paired with a primer corresponding to nucleotide nos. 11 to 32 were employed for the second amplification.

Nucleotide Sequence Determination

The PCR products were sequenced directly by an automated sequencer (ABI PRISM™310 Genetic Analyzer) with the PRISM Ready Reaction Dye Terminator Cycle Sequencing Kit (ABI Inc., Foster City, CA). Nucleotide sequences were analyzed for construction of phylogenetic tree using the unweighted pair group method with arithmetic means (UPGMA) provided by the GeneWorks software package (IntelliGenetics, Inc., Mountain View, CA).

Nucleotide Sequence Accession Numbers

The nucleotide sequence data reported in this paper appear in the DDBJ, EMBL and GenBank nucleotide sequence databases under the accession numbers AB008655 (K8 NSP5), AB008656 (AU-1 NSP5), AB008657 (O264 NSP5), AB008658 (M318 NSP5), AB008659 (512A NSP5), AB008660 (512B NSP5), AB008661 (KU NSP5), AB008662 (512C NSP5), AB008663 (470 NSP5), AB008664 (582 NSP5), AB008665 (O264 VP8*), AB008666 (O265 VP8*), AB008667 (M318 VP8*), AB008668 (512A VP8*), AB008669 (512B VP8*), AB008670 (208 VP4), AB008671 (208 VP7), AB008672 (208 VP6), and AB008673 (208 NSP4).

RESULTS

Detection of Rotaviruses

A total of 1,385 stool specimens were screened for rotavirus by PAGE of extracted RNA. The frequency of rotavirus detection was high; 56.5% (87/154) and 40.8% (502/1,231) of the specimens collected in 1994 and 1995, respectively, were positive for rotavirus. In both years, rotavirus was detected most frequently in infants and young children of 2 years or less with a peak at 10 to 14 months of age, and the male to female ratio was 1.7 (data not shown). The number of specimens with short RNA pattern was higher (67.8%) than that (26.4%) with long RNA pattern in 1994, while most of the specimens (99.2%; 498/502) showed long RNA patterns in 1995 (Table I). At least 10 specimens exhibited more than 11 RNA segments suggesting the occurrence of mixed infection and/or RNA rearrangement (Fig. 1, lanes 3–6). In addition, one specimen showed the RNA profile characteristic to group C rotavirus (Fig. 1, lane 7).

TABLE III. Correlation Between G Serotype and P Type of Human Rotaviruses in Wuhan, China, in 1994 and 1995 as Determined by ELISA and/or PCR

	G1	G2	G3	Multiple reaction	ND	Total
P1A[8][a]	119	0	22	4	3	148
P1A[8] + P1B[4]	6	0	4	5	0	15
P1B[4]	2	52	1	2	2	59
P3[9]	0	0	0	1	0	1
ND	60	0	3	0	3	66
Total	187	52	30	12	8	289

ND, could not be determined.
[a]P type of the samples was determined by PCR.

Examinations of Antigenic Specificity

Two hundred eighty-nine stool specimens (86 in 1994 and 203 in 1995) were examined for antigenic specificity. Subgroup specificity was determined by ELISA using subgroup I- and II-specific MAbs. The results are shown in Table II. In 1994, 58 specimens were sub-

TABLE IV. Specimens Showing Unusual Combinations Among RNA Pattern, Subgroup, G Serotype, and P Type

Specimen no.	RNA pattern	Subgroup	G serotype	P type
2	Long	I	G1	P1A
113	Long	I	G1	P1A
122	Short	II	G3	P1B
124	Short	I	G1	P1B

group I and only seven were subgroup II, while 136 samples were subgroup II and only two were subgroup I in 1995. Subgroup specificity of 68 specimens could not be determined since they were not reactive with either of subgroup I- or subgroup II-specific monoclonal antibodies. They might have the third subgroup specificity [Taniguchi et al., 1984; Steel and Alexander, 1988]. G serotype was also examined by ELISA using G serotype-specific MAbs. G serotype of 75.9% (211/278) of the specimens examined could be determined. In 1994, 6, 38, and 6 specimens were G1, G2, and G3 serotype, respectively. In 1995, in contrast, 144, 2, and 15 specimens were G1, G2, and G3 serotype, respectively. All the specimens whose G serotype could not be determined by ELISA-G serotyping were subjected to PCR-G serotyping. G serotype of most specimens (74.4%; 58/78) could be assigned by PCR. As a result, the G serotype of 96.5% (279/289) of the specimens were determined by ELISA and/or PCR.

P (VP4) type of these Chinese rotaviruses was also assigned by PCR. P1B[4] and P1A[8] types were predominant in 1994 and 1995, respectively. The correlation between G serotype and P type of the specimens is shown in Table III. By the examination of the specimens by RNA-PAGE, subgroup specificity, G serotype specificity and P type specificity, four specimens were found to have unusual combinations of characteristics (Table IV); two specimens (No.2 and No.113) showed long RNA-subgroup I-G1-P1A[8], one specimen (No.122) short RNA-subgroup II-G3-P1B[4], and one specimen (No.124) short RNA-subgroup I-G1-P1B[4].

Cultivation of Rotaviruses

Sixteen specimens were prepared for propagation in cell culture: four (Nos. 114, A512, A958, and A991) showing RNA profiles of mixed infection or possible rearrangement, four (Nos. 2, 113, 122, and 124) with unusual combinations of serological specificity and RNA pattern, and eight (Nos. 63, 94, 111, 126, 143, 145, A215, and A1015) with common properties. Only four strains (Nos. 114, A512, A958, and A991) were isolated in cell culture. Original stool specimens of all the four strains showed RNA profile of mixed infection or rearrangement, suggesting that in the process of adaptation to cell culture, there might be some complementation between the RNA segments from different strains. Since specimen No. 512 cultivated in MA-104 cells still had more than 11 RNA segments, 10 different clones were isolated by plaque purification. They were grouped into three distinct clones (512A to 512C) based on their RNA profiles (Fig. 2), showing the occurrence of reassortment during the replication in cell culture. However, no clone with apparent genome rearrangements was obtained. The properties of all the three clones, 512A, 512B and 512C, were long RNA-subgroup II-G3-P3[9].

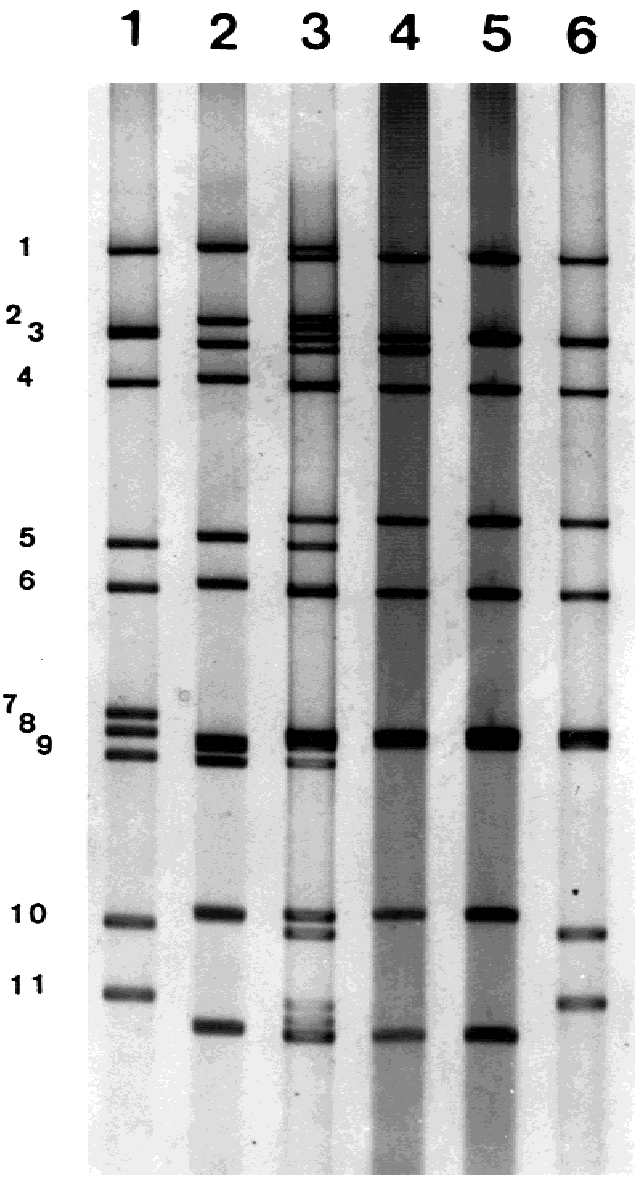

Fig. 2. RNA profile of different clones isolated from specimen No. 512 with more than 18 RNA segments which were resulted from mixed infection and/or rearrangement. Lane 1, strain KU; lane 2, strain K8; lane 3, specimen No. 512; lane 4, clone 512A; lane 5, clone 512B; lane 6, clone 512C.

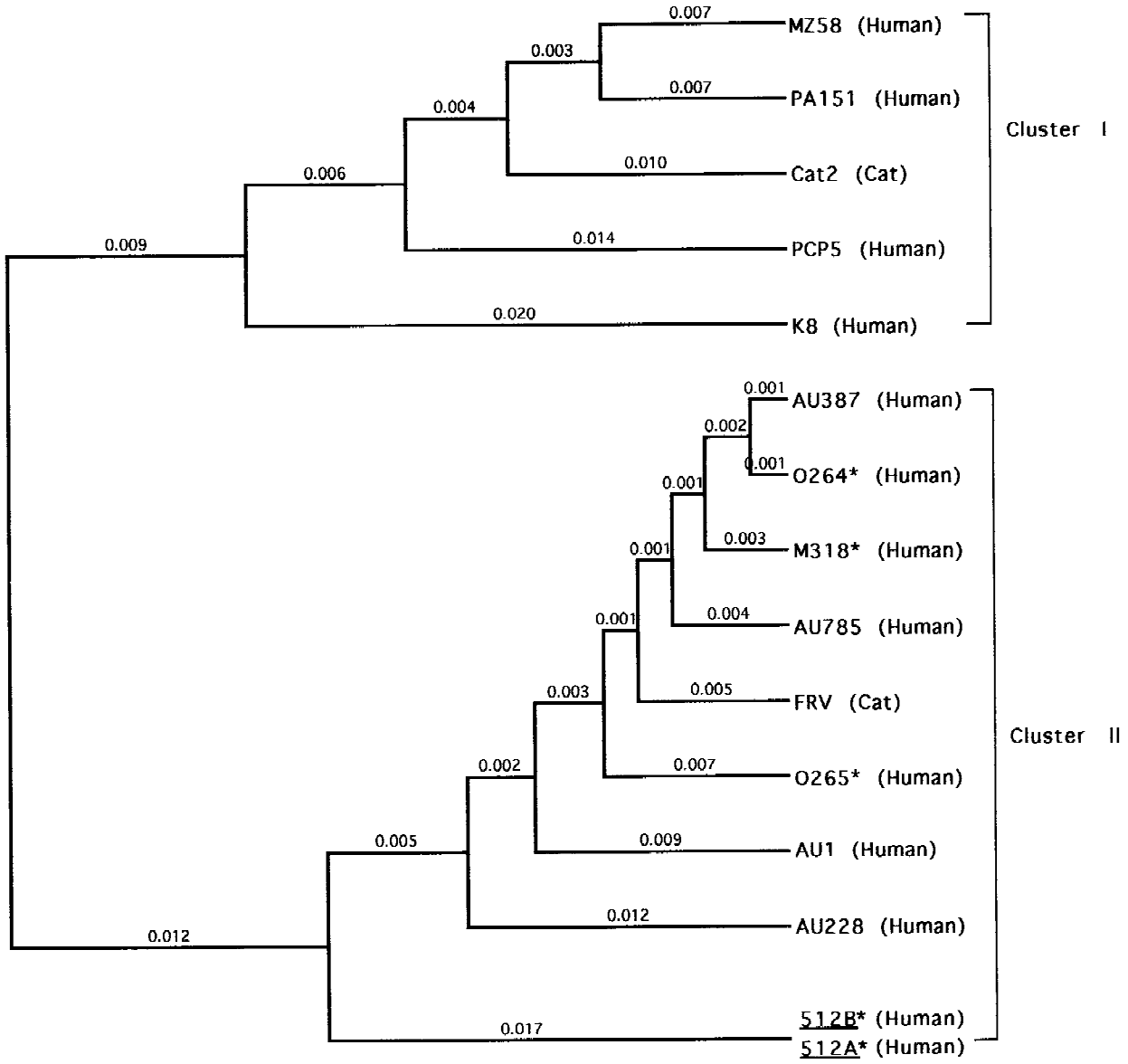

Fig. 3. Phylogenetic tree for the nucleotide sequences of the VP8* fragment of the VP4 gene of human and animal rotaviruses. The strains with an asterisk are the ones whose nucleotide sequence was determined in this study. Chinese strains characterized in this study are underlined.

Nucleotide Sequence Analysis

VP8* gene. The relative frequency of rotaviruses with P3[9] type VP4 represented by strains K8 and AU-1 is generally low [Gentsch et al., 1996]. In the present study, only one specimen (No. 512) was found to have VP4 with P3 type specificity. Two virus clones 512A and 512B isolated from specimen No.512 and three P3[9] strains (O264, O265, and M318) isolated in Japan [Urasawa et al., 1990b] were subjected to the nucleotide sequence determination of VP8* coding region (carrying the P type-specificity) of the VP4 gene. Based on the comparative analysis of nucleotide sequences, the phylogenetic tree was constructed for the VP8* fragment of the VP4 gene (Fig. 3). The presence of two clusters was confirmed as described by Nakagomi et al. [1993]; cluster I including strain K8 and cluster II including strain AU-1. Two Chinese virus clones 512A and 512B and three recent Japanese strains were included in the cluster II. The Chinese P3[9] viruses, however, were the least related to others among the strains forming the cluster II.

NSP5 gene. All of the above strains with P3 specificity showed a characteristic RNA profile; the mobility of gene 11 was faster in PAGE than those of common non-P3[9] human rotaviruses (Fig. 4), as described previously [Taniguchi et al., 1989; Urasawa et al., 1990b]. The nucleotide sequences of the gene 11 (NSP5 gene) of these strains having P3[9] specificity as well as two

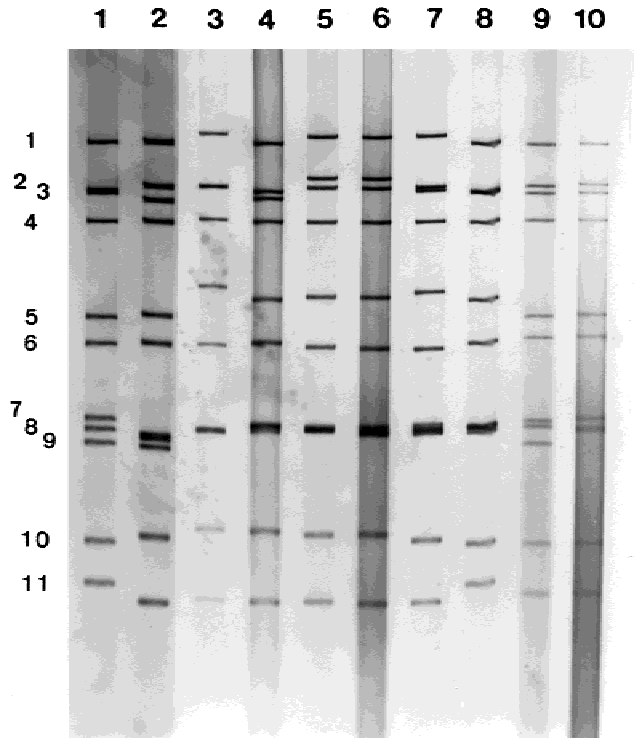

Fig. 4. RNA profiles of rotaviruses with P3 specificity and gene 11 (NSP5 gene) migrating faster than those of usual strains. Lane 1, strain KU; lane 2, strain K8; lane 3, strain AU1; lane 4, clone 512A; lane 5, strain M318; lane 6, strain O264; lane 7, strain O265; lane 8, clone 512C; lane 9, strain 582; lane 10, strain 470.

Chinese strains represented by strains 470 (G3-P1A[8]) and 582 (G1-P1A[8]) and one clone 512C derived from specimen No. 512 were determined. Based on the alignment of their sequences (Fig. 5), the phylogenetic tree was constructed (Fig. 6). All the human P3[9] strains including two clones 512A and 512B from Chinese strain 512 and three recently isolated Japanese strains as well as P3 reference strains K8 and AU-1 formed a cluster I together with simian and bovine rotaviruses, while three other Chinese clone or strains, 512C, 470 and 582, were the constituents of the other cluster II including strains Wa and KU which have usual migration of gene 11. Interestingly, NSP5 genes of the Chinese 470 and 582 strains were most closely related to those of porcine strains YM and OSU.

Characterization of Group C Rotavirus

Detection of only one group C rotavirus (strain Fuan) has been reported in a 4-year-old boy with diarrhea in China. However, the characterization of the strain was not carried out except for VP7 sequence analysis [Jiang et al., 1996]. In this study, one specimen (No. 208) showed the RNA profile characteristic to group C rotavirus. For comparing the genome of this group C rotavirus with those of the human group C strains reported worldwide, the nucleotide sequences of the genes encoding VP4, VP6, VP7 and NSP4 of strain 208 were determined. VP4 sequence of strain 208 had a high degree of identity (97.2 % and 96.7 % at nucleotide sequence and 97.7 % and 97.9 % at amino acid sequence, respectively) to those of two human group C rotaviruses, strains Bristol and Belem, reported so far. VP7 gene of strain 208 also showed a high level of homology (96.7 to 99.0% at nucleotide and 97.3 to 99.1% at amino acid levels) with those of all the human group C rotaviruses detected worldwide. Above all, the VP7 sequence of the strain 208 had a strong resemblance to that of Japanese E9301 strain [Jiang et al., 1996]. VP6 sequence of strain 208 was also very similar to those of other human group C rotavirus strains Belem, Bristol, and Preston (99.0% homology at amino acid level). The nucleotide sequence of NSP4 gene (encoding enterotoxin in group A rotaviruses [Ball et al., 1996]) of group C rotaviruses has been reported for only one strain (Bristol) [Deng et al., 1995]. Between NSP4 sequences of strains 208 and Bristol, 97.1% and 95.3 % identities at nucleotide and amino acid levels, respectively, were detected. Thus, a Chinese group C rotavirus 208 was found to be similar to other human group C rotaviruses.

DISCUSSION

Since the nationwide outbreaks of severe adult diarrhea due to group B rotavirus in China in 1982, detection of group B human rotavirus has been described only in China [Hung, 1988]. In contrast, only one group C rotavirus has been isolated in China [Jiang et al., 1996], although group C rotaviruses are thought to be endemic in humans throughout the world. In addition, a lamb rotavirus strain (Lp14) with G10 serotype and a new P type specificities was isolated in China [Shen et al., 1993], although G10 strains have been detected commonly in calves except for a few human rotaviruses [Urasawa et al., 1990b; Das et al., 1993]. These results suggest that characteristic ecological circumstances of rotavirus infections may exist in China. While close contact of humans with animals (especially livestock) is assumed to lead the emergence of reassortant rotaviruses as seen in the case of pandemic influenza viruses, extensive serological and genomic studies have not carried out in China. The purpose of this study was to examine properties of Chinese rotaviruses and to explore the unusual strains suggesting the possible human-animal transmission of rotavirus in China.

Zheng et al. [1989] serotyped 71 rotaviruses in Guangzhou and Foshan, China collected from 1982 to 1985. G1 was prevalent in the winter of 1982 and was replaced by G3 in the following year. The prevalence of G3 was markedly decreased in 1984 and G2 predominated during that year. In addition, Woods et al. [1992] examined 80 Chinese samples collected between 1982 and 1986; G1, G2, G3, and G4 were found in 38%, 23%, 11%, and 0% of samples, respectively. In the present study in which a large number of specimens were examined, a marked difference was found in G serotype distribution in Wuhan, China, between 1994 and 1995, i.e. a shift from G2 to G1. Such yearly change of G serotype distribution has also been found in Thailand

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512A    MSLSIDVTSLPSISSSIYKNESSSTTSTLSGKSIGRSEQYISPDAEAFSKYMLSKSPEDIGPSDSASNDPLTSCSNRSNAVKTNADVGVSMDSSTQSRPS 100
UK       Y                       A              V      S                           I          A       V      100
VMRI                F          A A           V  V                                  I          A       V      100
K8                                                                                            A              100
AU1                                                                                I          A      L       100
M318                                          R                                    I          A              100
264                                                                                I          A              100
265                                                                                I          A              100
SA11                 P T                                N                          I          A       A      100

512C                     F K                N   V   ID  N                          I          A      P       100
KU                       F                  N   V   ID  N                          I          A              100
470                      F                              N                          I          A              100
582                      F                              N                          I          A              100
YM                       F                              N                          I          A              100
V183          M          F                  N   V S I   N                          I          A              100
OSU                      F                              N                          I          A              100
Wa                       F                  N   V S I   N                   N      I     E    A              100

512A    SNVGCDQVDFSFNKGIKMSANLDSSVSISTHVKKEKSKNDHRSRKHYPKIEAESDSDDYVLDDSDSDDGKCKNCKYKKKYFALRMRMKQVAMQLIEDL 198
UK                      VN       I V  NSR        K      R                            R                    198
VMRI    I               VN       I V  NSR     G RK               E             R     R                    198
K8                      V             N                                              R          R         198
AU1                L    V             N                                              R                    198
M318               L    V             N                                              R                    198
264         Y      L    V             N                                              R                    198
265                L    V             N             TR              I                R                    198
SA11               L   LVK       I    DT      QN K      R                                                 198

512C           M   LT   NV  S   C     NQ      K -K      R   D   E       S                T                197
KU             M   L    NV  S   C     NQ      K -K      R   D   E                                         197
470            L   LT   NV      CI    DH      K -K      R   D   E                               R         197
582            L   LT   NV      CI    DH  GE  K -K      R   D   E                               R         197
YM                 LT   NV      CI    DH      K -K      R   D   E                                         197
V183    N      M   LT   NV  S   C     NH      K -K      R   D   E                                         197
OSU                LT   NV      C     DN      K -K      R   D   E                     RC    V             197
Wa             M   LT   NV  S VHVYQFQLTN      K -K      R   D  YE                                         197
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Fig. 5. Comparison of the deduced amino acid sequence of the NSP5s among human and animal rotaviruses. The sequence of 512A NSP5 is shown in its entirety; for the other NSP5s, only the differences from 512A are indicated. Dashes indicate deletions.

[Pongsuwanna et al., 1993], England [Noel et al., 1991], and other countries. G4 strains were not detected in the present study, although it is commonly found worldwide [Urasawa et al., 1989; Silberstein et al., 1995].

A high incidence of G5, G9, G10, G12 serotypes of human rotavirus has been reported in Brazil [Timenetsky et al., 1997], India [Ramachandran et al., 1996], Thailand [Urasawa et al., 1992] and the Philippines [Urasawa et al., 1990a], respectively. Although we could not detect such unusual G serotypes in China, strains with unusual combinations among RNA pattern, subgroup, G serotype, and P type were detected. Most of such unusual strains have been detected in the developing countries where close contact between humans and animals is suspected. A high prevalence of mixed infection has been described in developing countries [Timenetsky et al., 1997; Ahmed et al., 1991]. The mixed infections, resulting from a very frequent opportunity of infection, would also facilitate the occurrence of reassortment which may affect the rotavirus epidemiology.

The incidence of human P3 strains is generally low (1.1%)[Gentsch et al., 1996]. A single strain 512 (isolated clones 512A and 512B) with P3[9] specificity was detected in this study. Human strains with P3[9] specificity have been found to be highly related to feline rotaviruses by RNA-RNA hybridization assays [Nakagomi and Nakagomi, 1989]. Most human P3[9] strains carry G3 specificity. Strain K8 with G1-P3[9] specificity, therefore, seems to be a reassortant between feline or human strains with P3[9] and human strain with P1A[8] and G1 [Nakagomi et al., 1992]. The phylogenetic study based on the VP8* sequence associated with P serotype specificity revealed two clusters; cluster I comprising strain K8 and cluster II including strain AU-1. VP8* sequence of the Chinese P3[9] strains (512A and 512B) indicated that they belong to cluster II, though least related to other P3[9] strains in this cluster.

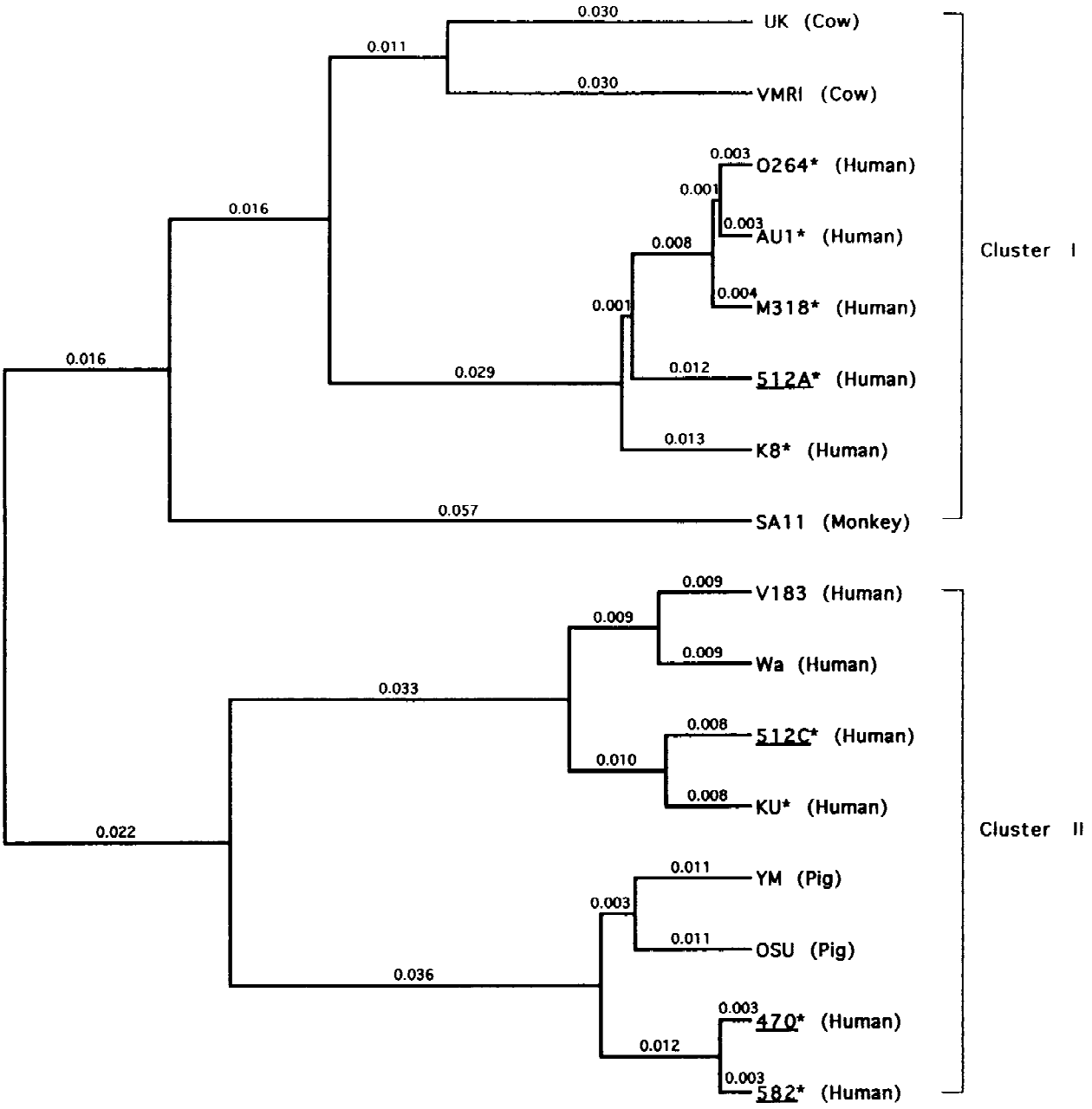

Fig. 6. Phylogenetic tree for the nucleotide sequences of the NSP5 genes of human and animal rotaviruses. The strains with an asterisk are the ones whose nucleotide sequence was determined in this study. Chinese strains characterized in this study are underlined.

The nucleotide sequences of NSP5 gene from the strains with P3[9] specificity were first determined in this study. The NSP5 genes of all the virus strains which exhibit faster mobility in PAGE constituted one cluster in the phylogenetic tree, and their total length (667 base pairs) was unexpectedly three base pairs more than that (664 base pairs) of the gene 11 with usual mobility. This is interesting since most animal strains have fast migrating gene 11 with 667 base pairs encoding 198 amino acids. It is of note that the P3[9] human strains commonly have the NSP5 sequence with 667 base pairs. In the phylogenetic analysis and overall genomic relatedness, porcine strains, which have subgroup I specificity and an NSP5 gene similarly migrating to human P1A[8] strains, are related to human rotaviruses with subgroup II-P1A[8] specificity. In view of rotavirus evolution, there might exist a genomic relatedness between humans and pigs, between humans and cows, and between humans and cats. It is interesting that VP4 gene of the strains with P3[9] specificity commonly has a deletion of one codon in the region encoding VP5* compared to non-P3[9] human

rotaviruses and an insertion of one codon in the VP8* coding region as found in the VP4 gene of animal rotaviruses [Taniguchi et al., 1989].

Among the group C rotaviruses including the two strains in China, VP7 sequence is highly conserved; more than 97.3% at the nucleotide sequence level and more than 98.5% at the amino acid sequence level. This implies the presence of a single G serotype in human group C rotaviruses, in contrast to the diversity of group A rotavirus G serotypes.

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